Specification

Proteinase K A3830

Solubility:	soluble (H_2O)
Product Code:	A3830
Product Name:	Proteinase K
Short Description:	delivery form: lyophilized
Specifications:	DNases/RNases: not detectable Activity/mg: min. 30 mAnsonU Appearance: white pH (1 %; H_2O; 20°C): 6.2 - 6.8
Hazard pictograms	
WGK:	1
Storage:	2-8°C
Signal Word:	Danger
GHS Symbols:	GHS07 GHS08
H Phrases:	H315 H319 H334 H335
P Phrases:	P280 P302+P352 P304+P340 P305+P351+P338

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Specification

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	P342+P311
Origin:	from *Tritirachium album*
M:	27000 g/mol
CAS:	39450-01-6
EINECS:	254-457-8
CS:	35079090
Index Nr.:	647-014-00-9

Comment

Proteinase K belongs to the familiy of subtilisin-like serine proteases. It has an endo- and exoproteolytic activity. Activated by calcium (1 - 5 mM), the enzyme digests proteins preferentially after hydrophobic amino acids (aliphatic, aromatic and other hydrophobic amino acids). Proteins will be completely digested, if the incubation time is long and the protease concentration high enough. Upon removal of the calcium ions, the stability of the enzyme is reduced, but the proteolytic activity remains (3). Proteinase K has two binding sites for $Ca^{2\+}$, which are located close to the active center, but are not directly involved in the catalytic mechanism. Removal of the $Ca^{2\+}$-ions reduces the catalytic activity of proteinase K by 80 %. The residual activity is sufficient to digest proteins, which usually contaminate nucleic acid preparations. Therefore, the digest with proteinase K for the purification of nucleic acids is performed in the presence of EDTA (inhibition of magnesium-dependent enzymes). Is the presence of $Ca^{2\+}$ required, $Ca^{2\+}$ is added up to a concentration of 1 mM and is removed by the addition of EGTA (pH 8.0; final conc. 2 mM) later on. The pH-optimum is at 8, but the enzyme is active over a wide pH-range (pH 4.3 - 12). An elevation of the reaction temperature from 37°C to 50 - 60°C may increase the activity several times, like the addition of 0.5 - 1 % SDS. Temperatures above 65°C, trichloroacetic acid or the serine protease-inhibitors AEBSF, PMSF or DFP inhibit the activity. Proteinase K will not be inhibited by EDTA (see ref. 2), urea (1 - 4 M), SDS, citrate, iodoacetic acid or, interestingly, by other serine protease inhibitors like TLCK and TPCK. In case that proteinase K has to be inactivated, make sure, that the temperature is not below 95°C and the time not shorter than 10 minutes. A TCA-precipitation is well suited too.Proteinase K is used for the destruction of proteins in cell lysates (tissue, cell culture cells) and for the release of nucleic acids, since it very effectively inactivates DNases and RNases. Some examples for applications: Purification of genomic DNA from bacteria (miniprep): Bacteria from a saturated liquid culture are lysed and proteins are removed by a digest with 100 µg/ml proteinase K for 1 h at 37°C (ref. 1 Suppl. 40 page 2.4.1); Whole-Mount *in situ* hybridization and determination of RNAs in vertebrate embryos and isolated organs: Digest of the sample with e. g. 10 µg/ml proteinase K for 15 minutes at room temperature; The period of the treatment and/or the concentration of teh enzyme has to be optimized (ref. 1 Suppl. 35 page 14.9.3); Prepration of DNA from cells or tissue for PCR: Cells or tissue are incubated over night at 50°C with 100 µg/ml proteinase K (ref. 1 Suppl. 17 page 15.3.1); Isolation of vaccinia virus DNA: Digest the virus in a suspension with 2 mg/ml proteinase K for 4 h at 37°C (ref. 1 Suppl. 43 page 16.17.8); Before the phenol extraction for the purification of nucleic acids is performed, a digest with proteinase K may be introduced (50 - 200 µg/ml final concentration; 37°C for 30 minutes in the presence of SDS; ref. 4). We recommend a working concentration between 10 - 100 µg/ml.**Stabiliy**\: Lyophilized proteinase K is stable at +4°C for at least 12 months. In solution, the stabiliy is approx. 6 - 12 months at +4°C to -20°C . Stock solutions (10 - 20 mg/ml) may be prepared in 10 mM $CaCl_2$ or 50 mM Tris · HCl, pH 8.0; 1 mM $CaCl_2$ or 50 % Glycerol; 20 mM Tris · HCl, pH 7.4; 1 mM $CaCl_2$ or 50 % Glycerol; 50 mM Tris · HCl, pH 8.0; 1 mM $CaCl_2$.

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Bibliography

(1)Ausubel, F.A., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. & Struhl, K. (eds.) (1995) *Current Protocols in Molecular Biology*. Greene Publishing & Wiley-Interscience, New York (2)Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning*\: A Laboratory Manual, 2nd Edition page B16. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. (3)Müller, A. *et al*. (1994) *J. Biol. Chem.* **269**, 23108-23111Crystall structure of calcium-free proteinase K with a resolution of 1.5 A. (4)Wallace, D.M. (1987) *Methods Enzymol.* **152**, 41-48Small and large scale phenol extractions.

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